Human Antibodies Promote Remyelination of Spinal Cord Lesions In a Model of Multiple Sclerosis

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ABSTRACT

A major goal in multiple sclerosis (MS) treatment is the promotion of central nervous system (CNS) remyelination. Ideally, the approach to enhance remyelination should be readily applicable, feasible and non-injurious. We have generated two human monoclonal antibodies (mAbs) that promoted remyelination in a viral model of MS. Both human mAbs promoted extensive CNS repair and were superior to treatment with polyclonal IgG, a treatment that has shown partial efficacy in MS. The target of the human mAbs appears to be CNS antigens, one on the surface of oligodendrocytes. Human mAb therapy to promote remyelination is readily applicable to clinical trials in demyelinating disease. The use of a human mAb with variable light and heavy chain domains similar to germline, increases the possibility that this approach may be effective and safe.

INTRODUCTION

Enhancement of remyelination and protection of axonal injury are important therapeutic goals in the treatment of inflammatory demyelinating CNS disorders such as MS. Remyelination in MS plaques can occur, but is limited[1-5] even though oligodendrocyte (OL) progenitors are present in the adult.[6,7] A number of potential therapeutic strategies to promote remyelination have been tested in experimental animals. Transplantation of OLs[8,9] or their progenitors[10] into previously demyelinated lesions produces new myelin and results in improved function.[11] Even though transplanted OL progenitors can migrate significant distances in the adult[12] or embryonic CNS[13,14] of myelin-deficient animals, unresolved issues remain concerning the survival of transplanted cells in the diseased adult CNS and the ability to target cells to areas of myelin pathology.[15]

The administration of growth or trophic factors induces the expansion of existing OL progenitors[16,17] or promotes mature OLs to dedifferentiate and subsequently reinitiate a program of myelination.[18-21] Administration of trophic factors via genetically engineered fibroblasts to the injured CNS promotes axonal sprouting and OL proliferation.[22] However, obstacles to *in vivo* trophic factor therapy remain, specifically determining the biologically relevant local factor concentration and the potential pleiotropic roles of most trophic factors administered at high concentrations.

Alternatively, our laboratory proposes to repair CNS pathology and enhance endogenous remyelination by using polyreactive CNS-binding immunoglobulins (Igs),[23] building on a natural reparative response that may already be upregulated following demyelination. Ig therapy can be rapidly adapted and tested as a treatment for human demyelinating disease.[24,25] The premise of our approach is that cells capable of remyelination—and the factors necessary to sustain their growth and differentiation—are present in the demyelinated CNS, but their capacity to produce myelin is limited.

We have used a virus-mediated model of demyelination to design an Ig-based therapy. When Theiler's murine encephalomyelitis virus (TMEV) is inoculated intracerebrally into susceptible strains of mice, TMEV induces immune-mediated progressive CNS demyelination clinically and pathologically similar to MS. A mouse mAb raised against spinal cord homogenate (SCH), designated SCH94.03, enhances remyelination in the TMEV model of demyelination.[26] SCH94.03 is a polyreactive, mouse IgMκ mAb that binds to the surface of OLs.[27] The light and heavy chain variable domains of SCH94.03 are encoded by Ig genes highly conserved when compared to germline sequences.[28] SCH94.03 also enhances the rate of spontaneous CNS remyelination following lysolecithin-induced demyelination[29] and decreases the severity and frequency of relapses in a model of experimental autoimmune encephalomyelitis (EAE).[30] Additional OL-binding mouse IgMκ mAbs, several of which are routine markers for the OL lineage and are coded for by germline sequences, promote CNS remyelination.[31,32]

Since polyreactive mouse IgM mAbs promote remyelination, we hypothesized that polyclonal human IgM, potentially enriched in polyreactive Abs, would be a more effective treatment of demyelinating disease than polyclonal human IgG, an established therapy for immune-mediated disorders.[33] Treatment of chronically TMEV-infected mice with polyclonal human IgM resulted in enhanced remyelination compared to polyclonal human IgG. Two human mAbs were also identified, using an antigen-independent strategy, which promoted remyelination to a degree equivalent to polyclonal human IgM. We suggest that human remyelination-promoting mAbs may be an easily implemented, effective therapy for human demyelinating disease.

METHODS

Sources of human antibodies and their isolation

Normal human IgM was purified from pooled plasma of more than 2500 healthy donors by modified Deutsch-Kistler-Nitschmann's ethanol fractionation procedure followed by octanoic acid precipitation and two successive ion-exchange chromatography steps as previously described.[34] The purity of IgM was more than 90% as confirmed by ELISA and SDS-PAGE. Pooled human IgG from healthy donors designated clinically as IVIg was purchased from Miles Inc. (Elkhart, IN).

Human serum samples were obtained from the dysproteinemia clinic under the direction of Dr. Robert A. Kyle, Mayo Clinic, and chosen solely by the presence of an Ig clonal peak of greater than 2 mg/ml. Sera were from 102 patients with a wide variety of conditions characterized by a monoclonal IgG or IgM spike in the serum, including Waldenstrom's macroglobulinemia, multiple myeloma, lymphoma, and monoclonal gammopathy of undetermined significance (MGUS). Sera were dialyzed against deionized water for 3 days. Euglobulinic precipitates were collected by centrifugation (14,000 rpm / 30 min) and dissolved in PBS. Solutions were cleared by centrifugation and chromatographed on Superose 6 column (Pharmacia, Upsala, Sweden). Fractions corresponding to IgM were pooled and analyzed by PAGE (12%). IgM concentrations were determined by gel staining with Cypro Orange (Molecular Probes, Eugene, OR) and subsequent scanning with a Storm 840 (Molecular Dynamics Inc., Sunnyvale, CA). IgM solutions were sterile filtered. Cell culture supernatants were concentrated by polyethylene glycol or ammonium sulfate precipitation and purified by gel filtration chromatography on a Superose-6 column. Anti-myelin basic protein (MBP) Ab was from Boehringer Mannhein (Indianapolis, IN) anti-glial fibrillary acidic protein (GFAP) was purchased from Sigma. The anti-sulfatide mAb O4[35] was a gift from Dr. Steven Pfeiffer.

Generation of Epstein-Barr virus immortalized B-cell lines

The B95-8 marmoset cell line was obtained from ATCC (#CRL 1612) for the growth and isolation of ebv. Cells were seeded at $1x10^6$ cells/ml in RPMI-10 and allowed to grow for 3 days before harvesting of the supernatant. The ebv-containing supernatant was filtered (0.45-μm), collected and cryopreserved. The supernatant generally contained 10^2-10^3 transforming units/ml. Human peripheral B cells for immortalization were collected from the blood of normal adults, adults with rheumatoid arthritis (AKJR), adults with MS (MSI), and from fetal cord blood. Blood was diluted 1:2 in PBS and underlayered with Ficoll-Hypaque. After centrifugation the interface was removed. Cells were washed once in PBS and then twice in HBSS and then diluted to $4x10^6$ cells/ml in RPMI-10. $1x10^7$ cells were incubated with 2.5 ml of ebv-supernatant for 2-hours at 37°C. The cell suspension was incubated in small wells for 2 weeks then transferred to a 25 cm^2 tissue culture flask and cultured for 3 weeks. An aliquot of the culture was cryopreserved, the remainder expanded and clonal cell lines isolated by limiting dilution.

ELISA assays

Abs were screened for their reactivity to mouse SCH as described.[26] SCH at 0.01 mg/ml was coated onto polystyrene microtiter plates in 0.1 M carbonate buffer, pH 9.5, for 18 hours at 4°C, and then washed three times with PBS. Coated plates were blocked with PBS containing 1% bovine serum albumin (BSA) for 1 hour at room temperature, and then incubated with the primary Ab diluted to 10 μg/ml in blocking buffer for 2 to 24 hours at room temperature. Plates were washed three times with PBS/0.05% Tween 20 and the bound Ab was then detected with biotinylated goat anti-IgM or IgG, followed by alkaline phosphatase conjugated to streptavidin (all from Jackson ImmunoResearch, West Grove, PA), with p-nitrophenylphosphate as chromogenic substrate. Absorbance of the reaction was measured at 405 nm.

Tissue slice immunocytochemistry

Mouse or rat pup cerebellum was used as the source of neural tissue for the immunocytochemical comparison of Ab binding patterns. The procedure was performed as described[36] with modifications. Fresh, unfixed tissue was embedded in 3% low melting point agarose and cut into 225 μm sagittal sections with a McIlwain Tissue Chopper (Gomshall, Surrey, UK). Sections were maintained at 4°C throughout the procedure. Slices were transferred into 48-well tissue culture plates containing HEPES-buffered EBSS (E/H) and blocked for 30 min to 1 hour in E/H with 5% BSA. Sections were incubated with the primary Ab at 10 μg/ml in E/H with 1% BSA for 2 to 3 hours at 4°C. Sections were washed three times in E/H and incubated with an appropriate fluorescently-conjugated secondary Ab (Jackson ImmunoResearch, West Grove, PA) in E/H with 1% BSA for 1 to 2 hours. Sections were washed three times in E/H, once in PBS, and then post-fixed with 4% paraformaldehyde/PBS for 30 min. Sections are washed three times with PBS and mounted on glass slides in 90% glycerin/PBS pH 8.6 with 2.5% 1,4-diazabicyclo[2.2.2]octane (Sigma, St. Louis, MO) to prevent photobleaching[37] and 10 μg/ml bisbenzimide (Sigma) for nuclear localization. Samples were viewed with an Olympus Provis epifluorescent microscope and imaged with a SPOT digital camera (Diagnostic Instruments Inc., Sterling Heights, MI).

Oligodendrocyte cell culture and immunocytochemistry

Cerebral hemispheres from P0-P2 Holtzman Sprague-Dawley rats were prepared for mixed primary glial cell culture as described.[38] Rat OL progenitors were isolated as described.[39] Live cell surface staining was performed at 4°C for 12 min on unfixed cells after blocking with E/H with 5% BSA. Intracellular staining was performed after fixation with 4% paraformaldehyde and permeabilization for 5 min with 0.1% Triton X-100. Primary Abs were detected using fluorescently-conjugated secondary Abs (Jackson ImmunoResearch). Cell monolayers on glass coverslips were

mounted in 90% glycerin/PBS with 2.5% 1,4-diazabicyclo[2.2.2]octane and viewed with an Olympus Provis epifluorescent microscope equipped with a SPOT digital camera. Adult human OLs were prepared from fresh human temporal lobe tissue from patients undergoing therapeutic resection for intractable epilepsy. Tissue did not contain the epileptic focus and was of normal cytoarchitecture when examined by the Mayo Clinic Department of Surgical Pathology. Prior to dissociation, human tissue was maintained in cold E/H containing 50 U/ml penicillin 50 μg/ml streptomycin (Sigma). Following enzymatic digestion with 0.1% trypsin (Sigma) and mechanical dissociation using both metal (210 μm) and nylon mesh (60 μm), human glial cells were separated from erythrocytes and myelin in a sucrose gradient as described.[40] Human glial cultures were grown on both poly-D-lysine (Sigma) and laminin or fibronectin (Gibco BRL, Grand Isle, NY) coated plastic multi-wells (Becton Dickenson, Franklin Lakes, NJ) or glass coverslips (Fisher Scientific, Pittsburgh, PA) in a defined media of DMEM/F12 supplemented with N2 (Life Technologies, Gaithersburg, MD), biotin (5 ng/ml, Sigma), 0.5 % FCS (Life Technologies) and 10 μg/ml PDGF (R and D Systems, Minneapolis, MN). Immunocytochemistry on human cultures was as described for rodent cultures.

Virus and animals

The Daniel's strain of TMEV was used for these experiments and was prepared as described previously.[41] Female SJL/J mice from the Jackson Laboratories (Bar Harbor, ME) were used after 1-week acclimation. Mice 4- to 6-weeks of age were injected intracerebrally with 2×10^5 plaque forming units (PFU) of TMEV in 10 μl volume. Intracerebral injection results in greater than 98% incidence of chronic viral infection with demyelination. Chronically infected animals used for remyelination experiments were generally 5 to 8 months postinfection. Animals with chronic demyelination received a single intraperitoneal (IP) bolus injection of purified Abs in PBS. Dosages were 1.0 mg of polyclonal human IgG or IgM and 0.5 mg of the human mAbs. Animals were killed 5 weeks following Ab treatment for morphologic assessment. The 5-week time point was chosen

because studies in toxic models of demyelination indicate that CNS remyelination is almost complete in this time period.[42]

Quantitation of spinal cord demyelination/remyelination

We developed methods to quantify the amount of spinal cord demyelination and remyelination in susceptible mice using plastic-embedded cross sections stained with 4% paraphenylenediamine (PPD) to visualize myelin. To obtain a representative sampling of the entire spinal cord, 1 μm thick cross sections were cut from every third serial 1 mm block, generating 10 to 12 cross sections that represent samples from the cervical, thoracic, lumbar, and sacral spinal cord. A 1-μm thick cross section was cut from 1 mm serial blocks. From each cross section the area of white matter, white matter pathology, OL remyelination, and SC remyelination were calculated using a Zeiss interactive digital analysis system (ZIDAS) and camera lucida attached to a Zeiss photomicroscope (Carl Zeiss Inc., Thornwood, NY). White matter was outlined using a 4x objective. The areas of white matter pathology, defined as regions of white matter with demyelination or remyelination, were then traced using a 10x objective. Regions of pathology often contained macrophage infiltration, inflammation, and little or no PPD stain.

In mice with extensive myelin repair, the original boundaries of the demyelinated area must be determined. As OL-mediated remyelination results in thinner myelin sheaths, original areas of demyelination were identified as having a lighter myelin stain. Areas of pathology that contain SC remyelination were defined as having increased space between individual axons. The sum of the areas of pathology containing primary demyelination with or without remyelination (OL or SC) was determined as a measure of total demyelination.

Following the assessment of total white matter and the area white matter pathology, regions with remyelination were traced. These regions were defined as having either OL or SC remyelination. OLs are able to remyelinate multiple axon fibers, and this type of remyelination results in thin myelin

sheaths compared to normally myelinated axons. In contrast, SCs can remyelinate only a single fiber, resulting in thicker myelin sheaths and increased space between axon fibers compared to fibers with OL remyelination. In addition, SC bodies and nuclei can be observed adjacent to the axons they have remyelinated. To accurately trace areas of remyelination within lesions, a 25x objective was used. After outlining all regions of interest, total areas were calculated for each mouse by summing all respective areas traced on each of 10 to 12 spinal cord sections per mouse.

The percentage of spinal cord pathology per mouse was obtained by dividing the total area of white matter pathology by the total area of white matter sampled. The percentage of spinal cord remyelination per mouse was obtained by dividing the area of OL or SC remyelination by the total area of white matter pathology.

We have estimated that 0.25 mm^2 of remyelination can range from approximately 25,700 axons remyelinated by OLs to 8400 axons remyelinated by SCs. Repeated measures of remyelinated areas from a mouse with extensive myelin repair revealed comparable values differing only by 1.5%. The area of white matter pathology can also be detected with similar accuracy. To determine the validity of using 10 cross sections as a representation of the remyelination throughout the entire spinal cord, a direct comparison was performed using 10 cross sections versus all 32 cross sections of a single chronically infected mouse. Using ten cross sections resulted in a percent area remyelination value of 47.7%; assaying all 32 cross sections resulted in a percent area remyelination value of 40.0%. Either value would have indicated significant remyelination in our assay.

Sequencing of human mAb light and heavy chain variable domains

For sequencing of the sera-derived human mAb 22, RNA was isolated from white blood cells and translated into cDNA. Primers 5'ATGCTGCTAAGAAGGATGATGCTAAGAAAGATGATGCTAA GAAAGATGCAGACAAGATCCTGACAGCA and 5'CTTGGGCTGACCTAGGACGGTC were used to clone the light chain variable domain. Primers 5'CAGGTGCAGCTGGAGGTGGAGG and 5'AGACGAGGGGGAAA

AGGGTT were used to clone the heavy chain variable domain. For sequencing of the human mAb MSI19D10 RNA was isolated from immortalized B-cells and translated into cDNA. Primers 5'GHHRTYBDGDTGASBCAGWSYCC and 5'CAACTGCTCATCAGATGGCG were used to clone the light chain variable domain. Primers 5'AGGTGCAGCTGSWGSACTCDGG and 5'AGACGAGGGGGAAAAGGGTT were used to clone the heavy chain variable domain.

RESULTS

Polyclonal human IgG and IgM promoted CNS remyelination in TMEV-infected mice

Clinical studies in MS indicate that intravenous immunoglobulin (IVIg) may be partially effective in stabilizing the disease course.[25] To determine if IVIg could promote remyelination in the TMEV model of MS, chronically infected mice were treated with a single bolus intraperitoneal injection of 1 mg of polyclonal human IgG. A single dose was administered to avoid anti-human immune responses. The total dose of human Ig was approximately 0.05 g/kg body weight, corresponding to one-eighth the total dose used for human IVIg treatment. An additional group of mice were treated with a single 1 mg bolus of polyclonal human IgM. Upon examination of the spinal cords 5 weeks after treatment, the percent area of OL remyelination in mice receiving either polyclonal human IgG or IgM (14.2% and 23.2%, respectively) was significantly higher than the spontaneous OL remyelination observed in the PBS-treated group ($p<0.05$ for IgG, $p<0.01$ for IgM). There were no statistically significant differences in the areas of white matter, areas of white matter pathology, or the percent areas of Schwann cell (SC) remyelination between either treatment group or the PBS control group (Table 1).

Treatment with polyclonal human IgM resulted in more OL remyelination than that observed in mice treated with polyclonal human IgG ($p=0.05$, Fig 1A, B). Approximately one quarter of the

total area of myelin pathology was remyelinated in mice treated with polyclonal human IgM, representing thousands of ensheathed axons. On average, 1 mm^2 within individual confluently remyelinated areas of pathology corresponded to 46,000 to 125,000 remyelinated axons. Therefore, the CNS remyelination produced following Ab treatment was extensive. Few inflammatory cells or macrophages were present (Fig 1B). In contrast, in mice treated with PBS, areas of myelin pathology contained few remyelinated axons (pathology not presented). Signs of active myelin destruction, consisting of inflammatory cells and macrophages were present. We concluded that polyclonal human IgM is superior to polyclonal human IgG in the ability to promote extensive CNS remyelination.

Human mAbs promoted CNS remyelination in TMEV-infected mice

All of the previously identified mouse mAbs that promote CNS remyelination bind to OLs.[27,31] As a first step in identifying candidate human mAbs for testing in the TMEV model we assayed human mAbs for the ability to bind to the surface of rat OLs presented in mixed primary culture. Our sources of human mAbs were serum-derived human monoclonal IgMs (sHIgMs), sera-derived human monoclonal IgGs (sHIgGs), and Epstein Barr virus-immortalized human peripheral B-cell clone derived IgMs (ebvHIgMs). None of the 50 sHIgGs bound to OLs, but six of the sHIgMs bound to the surface of morphologically mature rat OLs (Fig 2 B, C).

The six OL-binding sHIgMs and two sHIgMs that did not bind to OLs were tested in chronically TMEV-infected mice. Groups of five infected animals, each received a single bolus injection of 0.5 mg of human mAb. Among the OL-binding sHIgMs tested, treatment with sHIgM 22 resulted in the highest percent area of OL remyelination, whereas sHIgM 14 yielded no greater OL remyelination than the PBS treated group. The other four OL-binding sHIgMs promoted remyelination at low levels within these two extremes. A second set of chronically TMEV infected animals were treated with sHIgM 22 and sHIgM 14 to confirm the initial observation. The combined data are presented in Table 1. To test if any sHIgM, irrespective of antigen specificity, could promote

remyelination we studied two sHIgMs (sHIgM 1 and sHIgM 2) *in vivo* which demonstrated no immunoreactivity to OLs in culture. The percent area of remyelination following treatment with sHIgMs 1 and 2 was not significantly different from the PBS or sHIgM 14 treatment groups (Table 1).

In the case of the ebvHIgMs, we were limited to those B-cell clones that produced enough mAb necessary for a complete *in vivo* remyelination assay (~3 mg). Five ebvHIgMs were obtained and tested *in vivo*. As was observed with the sHIgMs tested, a range of OL remyelination scores resulted following mAb treatment and morphological evaluation. Treatment with ebvHIgM MSI19D10 resulted in the highest percent area of OL remyelination. EbvHIgM AKJR4 yielded no greater OL remyelination than treatment with PBS (Table 1). The other three ebvHIgMs promoted remyelination at low levels within these two extremes. Two separate groups of infected mice were treated with ebvHIgM MSI19D10 to confirm the initial observation.

In all treatment groups (Table 1) the areas of white matter, areas of white matter pathology, and percent areas of SC remyelination were not statistically different. The percent area of OL remyelination in the groups treated with sHIgMs 1, 2, and 14, and ebvHIgM AKJR4 (8.4%, 11.4%, 8.6%, and 4.2% respectively) were not statistically different from the PBS-treated control group (6.7%). sHIgM 22 promoted an average percent area of remyelination of 17% with a significance value of $p<0.05$ when compared to the spontaneous remyelination observed in the PBS-treated group. Treatment with ebvHIgM MSI19D10 resulted in the highest percent area of remyelination of any human mAb tested *in vivo* thus far (26.5%), with a significance of $p<0.001$ when compared to the PBS-treated group.

Characterization of human antibodies that promoted remyelination

Polyclonal human IgM, but not polyclonal human IgG, bound to CNS antigens

Several mouse IgMκ mAbs which promote remyelination bind to the surface of OLs and to internal cellular antigens.[23,31] Polyclonal human IgM, but not polyclonal human IgG, bound to the surface of a subpopulation of mature MBP-positive rat OLs in culture (Fig 2A); neither bound to intracellular antigens of OLs or astrocytes.

Unfixed neural tissue was immuno-labeled with polyclonal human IgG or IgM to determine whether either recognized CNS cells other than OLs. Polyclonal human IgG did not bind to slices of unfixed of rat cerebellum (Fig 3A). In contrast, polyclonal human IgM bound strongly to many cell populations (Fig 3B). Polyclonal human IgM reactivity was lost upon prior fixation.

The remyelination promoting mouse mAb SCH94.03 was identified by its affinity to SCH.[26] Polyclonal human IgM bound to SCH with twice the affinity of polyclonal human IgG. To exclude the possibility that polyclonal human IgG or IgM may have promoted remyelination by neutralizing virus, each was tested for reactivity to purified TMEV antigens by Western blotting. Neither Ab reacted with TMEV proteins; however, rabbit polyclonal Abs raised against TMEV reacted strongly to four virus capsid proteins.

We concluded that the reactivity of polyclonal human IgM with CNS antigens may explain its superior ability to promote remyelination.

Human mAbs bound to CNS antigens

Of the six sHIgMs that bound to the surface of OLs, five also bound to internal astrocyte antigens. Both sHIgM 22, which promoted remyelination, and sHIgM 14, which did not promote remyelination, bound to the surface of morphologically mature rat OLs (Fig 2 B, C). Neither ebvHIgM

MSI19D10, which promoted remyelination, or ebvHIgM AKJR4, which did not promote remyelination, bound to the surface of OLs. However, all four human mAbs bound to internal antigens (Fig 4) of cells expressing GFAP, a component of the astrocytic cytoskeleton.

Several mouse mAbs that are established markers of the OL lineage and promote remyelination in the TMEV model[31] bind to OLs, white matter, and neurons when incubated with unfixed slices of neonatal cerebellum.[36] All of the human mAbs in Table 1 bound to a slice of unfixed cerebellum (Fig 3). Only sHIgMs 14 and 22 bound SCH to any degree, yet each was only two-thirds as reactive as polyclonal human IgM. None of the human mAbs reacted with any TMEV viral proteins by Western blotting.

We concluded that an affinity for CNS antigens may be necessary, but not sufficient for a human mAb to promote remyelination. SHIgM 22 bound strongly to CNS antigens and rat OLs, whereas ebvHIgM MSI19D10 bound weakly CNS antigens and not at all to OLs. However, both human mAbs promoted remyelination. It is possible that OLs derived from early postnatal animals may not be the optimum source of OLs on which to study human mAb binding. We therefore, investigated whether human remyelination-promoting mAbs could bind to human OLs.

Human mAbs recognized human oligodendrocytes

If human mAbs are to be potentially successful in promoting remyelination in human demyelinating disease, their reactivity to surface antigens on human OLs may prove important in targeting to areas of human CNS pathology. When human glial cell cultures, established from adult human temporal lobe biopsies, were immuno-labeled at 1 week in culture morphologically immature sulfatide positive human OLs did not bind any of the human mAbs. However, after 3 weeks in culture, morphologically mature sulfatide positive human OLs co-labeled with sHIgM 22 (Fig 5). At 4 weeks in culture, virtually all sulfatide positive human OLs also bound sHIgM 22. No binding to human OLs

in culture was observed with polyclonal human IgG or IgM, sHIgM 14, or ebvHIgMs MSI19D10 and AKJR4 at any time tested.

The fact that only one of the two human mAbs that promote remyelination binds to human OLs underscores the possibility that the mechanism of action of the two human mAbs may be distinct.

Light and heavy chain variable domain sequences of human mAbs that promoted remyelination

Mouse IgMκ Abs which promote remyelination consist of light and heavy chain variable domains coded for by genetic sequences close to germline.[28,32] Therefore, the nucleotide sequences of the light and heavy chain variable domains of the human mAbs sHIgM22 and ebvHIgM MSI19D10 were compared with known human germline sequences of Ig variable regions using the international ImMunoGeneTics (IMGT) database[43] (Fig 6).

The closest germline sequence match for the variable heavy chain (Vh) sequence of ebvHIgM MSI19D10 was 83.6%. All differences in the sequence are located in the FR3 and CDR3 regions. In contrast, the sequence of the κ light chain variable region (Vκ) from ebvHIgM MSI19D10 completely matches a known human germline sequence. The closest germline sequence match for the Vh sequence of sHIgM 22 was 96.5%. The sequence of the Vκ from sHIgM 22 matches a known germline sequence at 97%.

DISCUSSION

In this study we demonstrated that human Abs promote CNS remyelination *in vivo*. More extensive remyelination was observed in the spinal cords of TMEV-infected mice following treatment with polyclonal human IgM than treatment with polyclonal human IgG. Of most importance, we identified two human IgM mAbs that consistently enhanced remyelination in the TMEV-mediated model of demyelination. One mAb was isolated from the serum of a Waldenstrom's macroglobulinemia patient and, of particular interest, the other was obtained from a peripheral B-cell isolated from an MS patient. Remyelination-promoting mAbs may be produced in the sera of individuals when confronted with CNS damage. Our ability to readily identify and isolate CNS antigen-binding, remyelination promoting mAbs from the human population lends support to the concept that these Abs are common among the B-cell repertoire and may function as modifiers in response to CNS injury.

Mouse IgMκ mAbs that promote spinal cord remyelination have a common affinity to OL surface antigens and genotypic and phenotypic features of natural autoantibodies.[27,28,31,32] Human Abs that promote remyelination may not all possess these characteristics. Although both polyclonal human IgG and IgM promoted remyelination only polyclonal human IgM bound to the surface of OLs. Human mAb sHIgM 22 was reactive with CNS and OL surface antigens, and the light and heavy chain variable domains were similar to germline. Human mAb ebvHIgM MSI19D10 reacted to CNS antigens, did not bind to OL surface antigens and the variable heavy chain domain sequences were somewhat divergent from germline. The distance of MSI19D10 from germline could be related to its relative absence of broad specificity. Alternatively, MSI19D10 may represent an as yet undescribed human germline sequence. Presently, sHIgM 22 has the greater potential for use in clinical trials, as a germline human mAb may present the least potential antigenicity.

The mechanism by which Igs promote remyelination remains to be elucidated. Since many of the remyelination-promoting mAbs bind to OLs and/or myelin, it is reasonable to hypothesize a direct effect on the recognized cells. There are examples of mAbs binding to and altering the biology of OLs in culture.[44-46] However, it is unlikely that each remyelination-promoting mAb functions directly through a common antigen or receptor. A more plausible role is that by targeting to myelin debris or damaged OLs, these mAbs may enhance the clearance of cellular debris from the demyelinated lesions, allowing the normal process of spontaneous CNS remyelination to progress. Remyelination-promoting mouse mAbs are also effective in three different models of demyelination: a viral-induced,[26] a T-cell–mediated,[30] and a toxin-induced model.[29] Perhaps the mechanism of action of polyclonal human IgG is primarily through immunomodulation,[33] whereas the action of polyclonal human IgM and sHIgM 22 is via targeting to OL antigens and/or myelin. No characteristic was completely predictive of an Ab's ability to promote remyelination. In fact, preliminary results suggest that certain OL-binding human mAbs inhibited remyelination *in vivo*, consistent with the observation that specific Abs reactive to surface antigens on OLs (i.e., myelin oligodendrocyte glycoprotein) enhance demyelination in EAE.[47] Ultimately, proof of an Ab's remyelinating potential and lack of pathogenicity requires *in vivo* testing.

Several double-blind, placebo-controlled trials with IVIg have shown some efficacy in MS.[25,48,49] We suggest that polyclonal human IgM, sHIgM 22, or ebvHIgM MSI19D10 may be more efficacious in the treatment of MS than polyclonal human IgG. Each Ab bound to neural tissue better than polyclonal human IgG and may be superior in targeting to areas of myelin destruction and facilitating the clearing of debris. Human mAbs that bind to OLs (sHIgM 22) may have the additional benefit of direct OL stimulation. Human mAbs can be produced free from potential pathogen infection and can be structurally altered to augment their effectiveness. In contrast to mouse mAbs or "humanized" mouse mAbs, human germline mAbs should result in minimal immune response and are readily applicable to human trials. Remyelination may be enhanced through the infusion of exogenous

beneficial mAbs or by upregulating endogenous B-cell populations secreting beneficial mAbs in patients with demyelinating disease.

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Table 1. CNS Remyelination by Human Antibodies

Treatment	No. of Mice	Area of White Matter (mm^2)	Area of Myelin Pathology (mm^2)	Area of CNS-Type Remyelination (mm^2)	Area of CNS-Type Remyelination (%)
Polyclonal Human IgG	10	8.60 ± 0.52	0.86 ± 0.10	0.13 ± 0.02	14.15 ± 2.38*
Polyclonal Human IgM	14	9.70 ± 0.43	1.21 ± 0.21	0.24 ± 0.04	23.2 ± 3.26†
sHIgM 1	4	9.34 ± 1.93	0.68 ± 0.07	0.03 ± 0.01	8.35 ± 3.73
sHIgM 2	4	8.78 ± 0.70	0.87 ± 0.12	0.09 ± 0.01	11.37 ± 1.30
sHIgM 14	7	10.68 ± 0.24	0.98 ± 0.09	0.08 ± 0.03	8.57 ± 2.51
sHIgM 22	8	10.55 ± 0.41	1.16 ± 0.22	0.19 ± 0.05	17.06 ± 3.42*
ebvHIgM MSI19D10	8	8.24 ± 0.40	0.90 ± 0.14	0.26 ± 0.07	26.47 ± 3.71‡
ebvHIgM AKJR4	4	8.70 ± 0.84	1.10 ± 0.15	0.05 ± 0.03	4.15 ± 1.98
PBS	7	9.78 ± 0.60	1.20 ± 0.22	0.06 ± 0.02	6.74 ± 1.80

Values represent the mean ± SEM. Statistics were done by using *t* test comparison of the percentage of area of CNS-type remyelination per area of white matter pathology in mice treated with human antibodies to those treated with PBS revealed * $p<0.05$; † $p<0.01$, ‡ $p<0.001$. Comparison of mice treated with polyclonal human IgG to those treated with polyclonal human IgM revealed $p=0.05$. There was no difference in the CNS-type remyelination between polyclonal human IgM, sHIgM 22 or ebvHIgM MSI19D10. Area of PNS-type Schwann cell remyelination ranged from 0 to 0.08 mm^2. This corresponded to 0.0 to 6.92 percent area of PNS-type Schwann cell remyelination as a function of myelin pathology. There was no difference in the PNS-type Schwann cell remyelination between groups.

FIGURE LEGENDS

Figure 1

White matter pathology of TMEV-infected mice following treatment with human polyclonal and monoclonal antibodies

Light photomicrographs of regions of white matter pathology within the spinal cords of SJL/J mice chronically infected with TMEV. Extensive OL remyelination, characterized by a dense packing of thin myelin sheaths in relation to axon diameter (arrow in A), was observed in mice after treatment with polyclonal human IgG (A), polyclonal human IgM (B), and human mAbs sHIgM 22 (E) and ebvHIgM MSI19D10 (G). In contrast, in mice treated with human mAbs sHIgM 1 (C), 2 (D), and 14 (F), and ebvHIgM AKJR4 (H), demyelination without significant OL remyelination was observed, along with inflammatory cells and macrophages (arrow in C), signs of active myelin destruction. Individual areas of white matter pathology in 4 of 8 animals treated with sHIgM 22 and 7 of 8 animals treated with ebvHIgM MSI19D10 contained at least one area of complete repair consisting of nearly confluent remyelinated axons. In contrast, no area of white matter pathology in mice treated with sHIgM 1, 2 or 14, ebvHIgM AKJR4, or PBS consisted of nearly confluent remyelinated axons. Scale bar=100 μm.

Figure 2

Human antibodies bound to the surface of rat oligodendrocytes in culture

By immunocytochemistry polyclonal human IgM, and human mAbs, sHIgM 14 and sHIgM 22, bound to unfixed rat OLs in culture. Polyclonal human IgM bound to the cell body and the elaborate membrane extensions of mature, differentiated, myelin basic protein positive rat OLs (A).

sHIgM 14 (B) and sHIgM 22 (C) bound to the surface of less mature myelin basic protein positive rat OLs. Both human monoclonal Abs highlight the OL perikaryon, but the proximal process extensions are revealed to varying degrees by punctate immunolabeling. sHIgM 22 OL reactivity was eventually lost as rat primary mixed glial cultures matured, whereas sHIgM14 continued to weakly bind to rat OLs. Scale bar=50 μm.

Figure 3

Human polyclonal and monoclonal antibodies bound to CNS antigens

Indirect immunohistocytochemistry on unfixed slices of postnatal day 6 rat cerebellum. Human Abs bound to a variety of cell populations and structures. Polyclonal human IgG (A) bound at background levels to most structures within the cerebellum. Polyclonal human IgM (B) bound to myelin in the central white matter of the folia, Purkinje cell bodies, and small cells within the granular and molecular layer. sHIgM 1 (C) bound primarily to the cytoskeleton of astrocytes overlying the central white matter of the folia and weakly to many other scattered cells. sHIgM 2 (D) bound to cells of the granular layer, fibers traversing the central white matter of the folia, and cells within the central white matter with the morphology consistent with being microglia and OLs. Purkinje cells were also weakly identified but their dendritic arbors were not. sHIgM 22 (E) bound to many structures within CNS, including the central white matter, OLs, Purkinje cells, and molecular layer cells. sHIgM 22 also recognized damaged astrocytes overlying the central white matter of the folia, and weakly, but uniformly, the surface of granule cells. sHIgM 14 (F) bound to cells of the granular layer and Purkinje cells and their dendritic arbors. The central white matter of the folia was largely devoid of label. ebvHIgM MSI19D10 G) bound weakly to cerebellar slices, but consistently labeled Purkinje cells and the internal granular layer. ebvHIgM AKJR4 (H) bound primarily to the internal granular layer and, less so, to small cells in the molecular layer and potential microglia in the central white matter. Scale

bars=100 μm in A-G and 250 μm in H. Abbreviations in figure panels: *wm*, white matter; *pc*, Purkinje cells; *gl*, granular layer; *ml*, molecular layer.

Figure 4

Human monoclonal antibodies bound to internal astrocytic antigens

Immunocytochemical label of fixed/detergent permeablilized rat astrocytes in culture. sHIgM 14 (A) sHIgM 22 (B), and ebvHIgMs MSI19D10 (C) and AKJR4 (D) bound to internal epitopes in cells that expressed (GFAP), a cytoskeletal antigen widely used as a marker for astrocytes. No reactivity to internal astrocytic epitopes was observed with polyclonal human IgG, polyclonal human IgM, sHIgM 1, or sHIgM 2. Each human mAb bound with varying paterns and intensities suggesting that each mAb recognizes different astrocytic epitopes. N: cell nucleus. Scale bar=50 μm

Figure 5

Human monoclonal antibody sHIgM 22 bound to human oligodendrocytes in culture

Immunocytochemical label of cultured human glial cells maintained in serum-free media for 3 weeks. At this time in culture human OLs differentiated into myelin basic protein positive cells that elaborated long, complex process extensions. Human OLs labeled live with O4 mAb,[35] to localize sulfatide to the surface (A), also bound sHIgM 22 (B). Polyclonal human IgG, polyclonal human IgM, and human mAbs sHIgM 14, and ebvHIgM MSI19D10 did not label human OLs at any time point examined. Asterisk indicates cell body. Scale bar=50 μm.

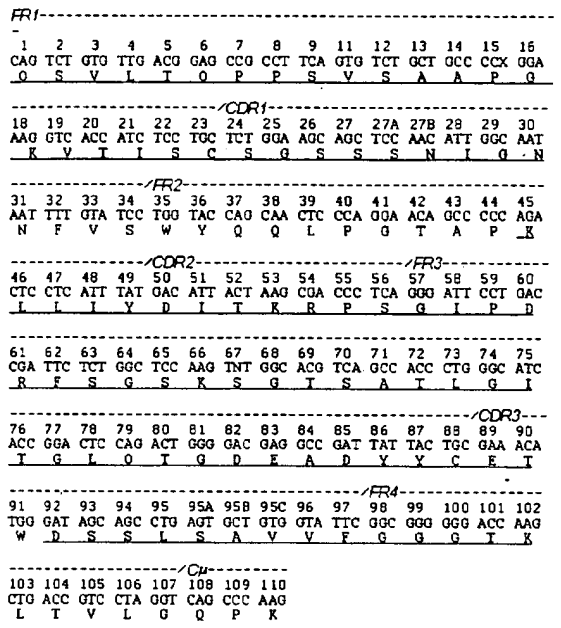
Figure 6

Light and heavy chain variable domain genetic sequences of remyelination promoting human monoclonal antibodies

Nucleotide and translated amino acid sequences were aligned according to the numbering system of human variable region chain sequences[50]. The sHIgM 22 light chain variable region (A) belonged to the λ subgroup I of the human light chain variable regions. The sHIgM 22 heavy chain variable region (B) belonged to subgroup III of the human heavy chain variable regions. The ebvHIgM MSI19D10 variable κ light chain region (C) was identical to subgroup IV κ human light chain variable region germline sequence and Jκ3 with the exception of the L at position 96. The ebvHIgM MSI19D10 variable heavy chain region (D) belonged to subgroup IV of the human heavy chain variable regions. Amino acids 3 and 5 are ambiguous. The amino acids at these positions match the human germline sequences for this family. Underlined amino acids have been confirmed by protein sequencing. Ref: IMGT, the international ImMunoGeneTics database http://imgt.cnusc.fr:8104 (Initiator and coordinator: Marie-Paule Lefranc, Montpellier, France, lefranc@ligm.igh.cnrs.fr).

A

sHIgM 22 light chain variable region

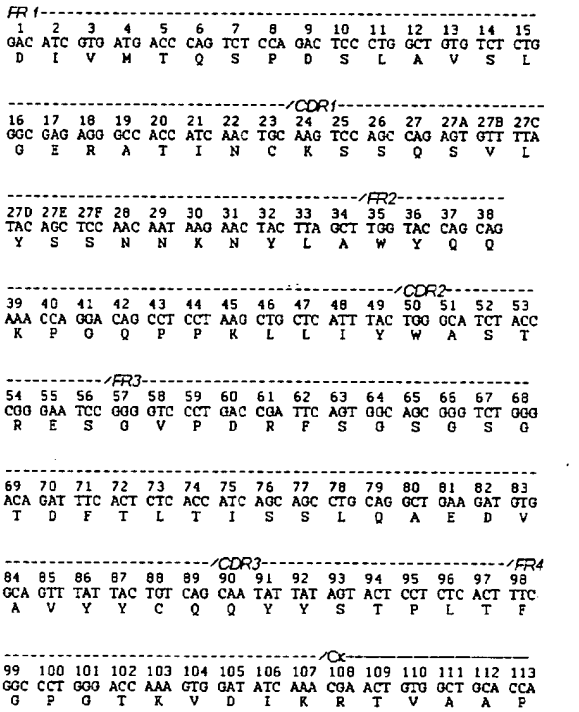


B

sHIgM 22 heavy chain variable region

C

ebvHIgM MSI19D10 light chain variable region



D

ebvHIgM MSI19D10 heavy chain variable region